

Comparison of complete mitochondrial genome sequences in the *Aporrectodea caliginosa* species group (Annelida, Crassiclitellata, Lumbricidae)

Csaba Csuzdi^{1*}, Jachoon Koo^{2*}, Yong Hong³

¹ Kenderesi str. 39, Piliscsaba, Hungary

² Division of Science Education and Institute of Fusion Science, College of Education, Jeonbuk National University, Jeonju 54896, Republic of Korea

³ Department of Agricultural Biology, College of Agriculture & Life Science, Jeonbuk National University, Jeonju 54896, Republic of Korea

Corresponding author: Yong Hong (yonghong@jbnu.ac.kr)

Abstract

We present for the first time the complete mitochondrial genomes (mt genomes) of the earthworms *Aporrectodea caliginosa* and *Ap. trapezoides* (Clitellata, Megadrili) collected in Hungary and Korea, respectively.

The complete mt genome of *Ap. trapezoides* comprised 15,014 base pairs. Lengths of the three complete *Ap. caliginosa* mt genomes varied between 15,090 and 15,123 bp. All four mt genomes contained 13 protein-coding genes (PCGs), two rRNA genes, 22 tRNA genes, and one major non-coding control region. These mt genome arrangements are identical to those observed in the mt genomes of most earthworms, and all the 37 genes are transcribed from the same directional strand. All 13 PCGs had the same ATG start codon. Most of the PCGs end with TAA or TAG, whereas the remaining end with an incomplete stop codon, T. Stop codons were consistent in the PCGs throughout the mt genomes, except *Ap. caliginosa* 5, which contains a TAG stop codon in ND5 instead of the TAA found in the other samples. Both species' genomes showed biased base composition, with 63.5% AT and 36.4% GC content in *Ap. trapezoides* and 62.8% and 37.2% in *Ap. caliginosa*. Phylogenetic analysis of the mt genomes corroborated the monophyly of the family Lumbricidae and the close relationship between *Ap. trapezoides* and *Ap. caliginosa* species pairs. The available *Ap. tuberculata* sequences were embedded between the *Ap. caliginosa* samples, thereby supporting the synonymy of the two names.

Key words: *Aporrectodea caliginosa*, *Aporrectodea trapezoides*, Lumbricidae, mitochondrial genome, phylogeny

Introduction

Aporrectodea caliginosa was described as *Enterion caliginosum* Savigny, 1826 and placed in Savigny's "Tribu" No. 1 (Savigny 1826: 179). The "Tribu" No. 1 is defined as follows: "Les soies sont rapprochées par paires. La ceinture a de chaque côté deux pores qui correspondent chacun à un seul segment, et qui, si l'on compte celui qui les sépare, comprennent les trois pénultièmes" [The setae paired. The girdle (clitellum) has two pores (tubercles) on each side, each of



Academic editor: Samuel James

Received: 16 December 2024

Accepted: 6 February 2025

Published: 13 March 2025

ZooBank: <https://zoobank.org/BEFE7D67-93C1-4608-97C2-A74E260B6080>

Citation: Csuzdi C, Koo J, Hong Y (2025) Comparison of complete mitochondrial genome sequences in the *Aporrectodea caliginosa* species group (Annelida, Crassiclitellata, Lumbricidae). ZooKeys 1231: 293–310. <https://doi.org/10.3897/zookeys.1231.144623>

Copyright: © Csaba Csuzdi et al.
This is an open access article distributed under terms of the Creative Commons Attribution License ([Attribution 4.0 International – CC BY 4.0](https://creativecommons.org/licenses/by/4.0/)).

* These authors contributed equally.

which corresponds to a single segment, and which, if the one between them is counted, comprise the three penultimate segments]. The species *caliginosum* was defined as “La ceinture, de huit segmens, finit avec le trente-quatrième du corps” [The clitellum, of eight segments, ending with the thirty-fourth of the body]. The diagnosis of *E. caliginosum* in modern terms according to Cain (1955: 482–483) is as follows: “Male pores with conspicuous lips, apertures on segment 15; setae paired; clitellum on 27–34; tubercula pubertatis on 31 and 33, two pairs of spermathecae opening on the ventral surface; four pairs of seminal vesicles; coelomic fluid not colored.”

The identity of *Enterion caliginosum* has provoked a long and continuing debate (Blakemore 2008). Gates (1972a, 1972b) and other North American authors (e.g. Reynolds 1977, 2022) regarded Savigny’s definition as inadequate and used the junior synonym name *Allolobophora turgida* Eisen, 1873 because Tétry (1937) erroneously cited the position of the tubercles as being on segment 31, 32 when revising Savigny’s material housed in the Muséum national d’Histoire naturelle in Paris. However, Tétry (1937) did not find the type material of *E. caliginosum* and Savigny (1826) never wrote of this (Cain 1955). As *E. caliginosum* is published according to ICZN Article 11 and its diagnosis satisfies Article 13, it is an available and valid senior name for this taxon.

Later, several synonyms (e.g. *Lumbricus lividus* Templeton, 1836; *Allolobophora similis* Friend, 1910 (see Blakemore 2008: 526 for a complete synonym list) and closely related taxa were described, such as *Lumbricus trapezoides* Dugés, 1828; *Allolobophora nocturna* Evans, 1946; *Allolobophora turgida* var. *tuberculata* (Eisen 1874). In addition, the latter three taxa, which together with *Ap. caliginosa*, form the *Ap. caliginosa* species group (Blakemore 2002), have been treated differently by different authors. For instance, Pop (1949), Zicsi (1982), and Csuzdi and Zicsi (2003) regarded all three species names as synonyms of *Ap. caliginosa*. Gates (1972a) and Reynolds (1977, 2022) considered *Ap. trapezoides*, *Ap. tuberculata*, and *Ap. nocturna* independent taxa from *Ap. caliginosa* (syn. *Ap. turgida*). Mršić (1991) considered *Ap. tuberculata* as a synonym for *Ap. caliginosa*, *Ap. trapezoides* as a subspecies of *Ap. caliginosa*, and *Ap. nocturna* as a valid species. Briones (1996) reviewed the *Ap. caliginosa* species group and concluded that there were only two valid taxa, *Ap. caliginosa* and *Ap. trapezoides*. However, the author did not comment on the status of *Ap. nocturna* owing to a lack of sufficient material.

To further complicate this issue, Bouché (1972) distinguished three subspecies within *Ap. caliginosa*, along with several invalid infrasubspecific taxa (varieties), that he placed in his genus *Nicodrilus* Bouché, 1972, a junior synonym of *Aporrectodea* Örley, 1885 (Sims and Gerard 1985).

The *Ap. caliginosa* species group is one of the most molecularly studied earthworm taxa. Early multigene phylogenetic analyses (Pérez-Losada et al. 2009; Fernández et al. 2011, 2012, 2013; Briard et al. 2012) revealed high genetic variability in the species group and the presence of several highly divergent cryptic lineages in different constituent taxa. *Aporrectodea tuberculata* with *Ap. caliginosa* form a well-separated clade from the other taxa in this complex. However, *Ap. caliginosa* was paraphyletic without *Ap. tuberculata*, and *Ap. trapezoides* comprised two highly separated clades and appeared to be polyphyletic, as clade I is more closely related to the clade comprising *Ap. nocturna*, *Ap. giardi*

(valid name *Ap. terrestris*), and *Ap. longa*. Later studies either corroborated these results (Shekhovtsov et al. 2017; Marchán et al. 2020) or found *Ap. caliginosa* polyphyletic (Shekhovtsov et al. 2016; Latif et al. 2020).

Over the past few years increasing number of complete mitochondrial genomes (mt genomes) have been published, facilitating the differentiation of closely related species (Zhang et al. 2016; Shekhovtsov et al. 2020; Csuzdi et al. 2022). Recently, Zhao et al. (2022) reported several nearly complete lumbricid mt genomes, including one *Ap. trapezoides* and six *Ap. tuberculata* sequences. Interestingly, one of the reported *Ap. tuberculata* sequences is registered as *Ap. caliginosa* in the GenBank (sample 420Ra, accession number [NC_066400](#)).

Herein, we report the complete mt genomes of the Korean *Ap. trapezoides* and three Hungarian *Ap. caliginosa* specimens, as well as compare these data to the published complete or nearly complete mt genomes of the *Ap. caliginosa* species group to help solve this continuing taxonomic issue.

Material and methods

Sample preparation and DNA extraction

Adult *Ap. trapezoides* were collected from a farm in Seongsu-myeon, Imsil-gun, Jeollabuk-do, Korea (33°41'23.80"N, 126°38'33.67"E; 40 m a.s.l.) on March 28, 2021 and preserved in 99% ethanol until DNA extraction. *Ap. caliginosa* specimens were collected in a deciduous forest patch near Szendehely, Hungary (47°52'32.7"N, 19°6'16.6"E) on May 4, 2021, and preserved in 96% ethanol until DNA extraction. Voucher specimens of each species were deposited at Jeonbuk National University, Jeonju City, Korea. Total genomic DNA was prepared from a small portion of the body segments of a single adult earthworm using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The remaining tissue was preserved at −20 °C in 90% ethanol.

TruSeq DNA library construction

The sequencing library was prepared by random fragmentation of genomic DNA, followed by 5' and 3' adapter ligations using the Illumina TruSeq DNA Nano Library Prep Kit according to the manufacturer's instructions (Illumina Inc., San Diego, CA, USA). The resulting libraries were quantified through a qPCR-based assay using the KAPA Library Quantification Kit for Illumina sequencing platforms, according to the manufacturer's instructions (Kapa Biosystems, Woburn, MA, USA). Libraries were qualified using the Agilent Technologies 2200 TapeStation (Agilent Technologies, Santa Clara, CA, USA).

DNA sequencing, assembly, and annotation

Paired-end (2×150 base pairs [bp]) sequencing was performed using the Illumina HiSeq-X platform (Illumina Inc.) at Macrogen Inc. (Seoul, Korea). To reduce bias in the analysis, adapter trimming and quality filtering were performed using Trimmomatic v. 0.36 (Bolger et al. 2014). *De novo* assembly of raw sequencing reads was performed using various *k*-mer lengths in SPAdes

v. 3.13.0 (Bankevich et al. 2012). Mitochondrial contigs were assembled into a single contig using BLASTN alignment (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>) against the *Lumbricus terrestris* mitogenome (GenBank accession number NC_001673) as the reference sequence. Annotation and visualization of mt genomes were performed using the online MITOS software (Donath et al. 2019), and manual curation was performed using BLAST searches in the NCBI database for various earthworm mt genomes (Table 1). A comparative map of the mt genomes was created using Geneious Prime 2024 software. The *cox1* sequence was used as an anchor for the linearized mt genome maps. The annotated complete genome sequences were registered in GenBank under the accession numbers PQ572750–PQ572753.

Phylogenetic analyses

To check the identity of the morphologically identified specimens, the *cox1* barcoding regions were analyzed with barcodes selected and downloaded from the BOLD v. 4 database (Ratnasingham and Hebert 2013) using the IQ-tree web server with default settings and suggested TPM2u+F+I+G4 substitution model.

To clarify the phylogenetic positions of the two taxa, complete or nearly complete mitogenomes were obtained from GenBank, comprising 26 species of Megascolecidae and 12 species of Lumbricidae. *Pontoscolex corethrus* (Müller, 1857) from the Rhinodrilidae family and *Drawida japonica* (Michaelsen, 1892) from the exquisiclitellate family Moniligastridae were used as outgroups. Phylogenetic analysis was conducted based on two datasets, a complete set of 37 mitochondrial genes and subset comprising only 13 protein-coding genes (PCGs), using maximum likelihood (ML) and Bayesian (BI) methods. Sequences for each gene from the 40 earthworm species (Table 1) were individually aligned using MAFFT v. 7 (Kato and Standley 2013) and trimmed using TrimAl v. 1.2 (Capella-Gutiérrez et al. 2009). The trimmed sequences were concatenated into a single multi-gene alignment. The best-fit substitution model for each dataset was selected using ModelFinder (Kalyaanamoorthy et al. 2017) integrated within the IQ-TREE (Nguyen et al. 2014) based on the Bayesian Information Criterion (Schwarz 1978). The ML phylogenetic trees were constructed using IQ-TREE for both datasets by applying the selected GTR+F+I+R5 model. In addition, a partitioned ML analysis was also performed on the 13 PCG dataset using the best-fit models TIM2+F+R7 for *atp6+atp8+nd2+nd6* genes, GTR+F+I+G4 for *cox1+cox2+cox3+cytb* genes and TIM2+F+R10 for *nd1+nd3+nd4+nd4L+nd5* genes. Branch support was assessed using 5,000 ultrafast bootstrap replicates (Hoang et al. 2018). Bayesian analysis was performed using MrBayes v. 3.2.7 (Ronquist et al. 2012) with two independent runs set to 10 million generations each and sampling every 1000th generation (10,000 trees). Twenty-five percent of the trees were discarded as burn-in, and the remaining trees were combined and summarized into a 50% majority-rule consensus tree.

Estimation of the evolutionary divergences between the analyzed *Ap. caliginosa* group sequences were conducted in MEGA X (Kumar et al. 2018) using the uncorrected *p*-distance. The analyzed matrix was 14,681 bp long and contained only coding regions.

Table 1. List of Megadrili mitogenomes used in this study.

Family	Taxa	Genbank No.	Total length (bp)	Reference
Megascolecidae	<i>Amyntas aspergillus</i>	KJ830749	15,115	Zhang et al. 2016
	<i>Amyntas carnosus</i>	KT429008	15,160	Zhang et al. 2016
	<i>Amyntas corticis</i>	KM199290	15,126	Zhang et al. 2015
	<i>Amyntas cucullatus</i>	KT429012	15,122	Zhang et al. 2016
	<i>Amyntas gracilis</i>	KP688582	15,161	Zhang et al. 2015
	<i>Amyntas hupeiensis</i>	KT429009	15,069	Zhang et al. 2016
	<i>Amyntas jiriensis</i>	KT783537	15,151	Hong et al. 2017
	<i>Amyntas longisiphonus</i>	KM199289	15,176	Zhang et al. 2015
	<i>Amyntas moniliatus</i>	KT429020	15,133	Zhang et al. 2016
	<i>Amyntas morrisoni</i>	KT429011	15,026	Zhang et al. 2016
	<i>Amyntas pectiniferus</i>	KT429018	15,188	Zhang et al. 2016
	<i>Amyntas robustus</i>	KT429019	15,013	Zhang et al. 2016
	<i>Amyntas seungpanensis</i>	OL321943	15,085	Kim and Hong 2022
	<i>Amyntas</i> sp. 1	KT429010	15,131	unpublished
	<i>Amyntas</i> sp. 2 JS-2012	KT429007	15,159	unpublished
	<i>Amyntas</i> sp. 2	KT429014	15,086	unpublished
	<i>Amyntas</i> sp. 3	KT429013	15,152	unpublished
	<i>Amyntas triastriatus</i>	KT429016	15,160	Zhang et al. 2016
	<i>Amyntas yunoshimensis</i>	LC573969	15,109	Seto et al. 2021
	<i>Duplodicrodrilus schmardae</i>	KT429015	15,156	Zhang et al. 2016
	<i>Metaphire californica</i>	KP688581	15,147	Zhang et al. 2015
	<i>Metaphire guillelmi</i>	KT429017	15,174	Zhang et al. 2016
	<i>Metaphire hilgendorfi</i>	LC573968	15,186	Seto et al. 2021
	<i>Metaphire vulgaris</i>	KJ137279	15,061	Zhang et al. 2014
	<i>Perionyx excavatus</i>	EF494507	15,083	unpublished
	<i>Tonoscolex birmanicus</i>	KF425518	15,170	Wang et al. 2015
Lumbricidae	<i>Aporrectodea rosea</i>	MK573632	15,086	Shekhovtsov and Peltek 2019
	<i>Aporrectodea caliginosa</i>	PQ572750	15,090	this study
		PQ572751	15111	this study
		PQ572752	15123	this study
		CM035405	15,120	unpublished
		NC_066400	15,089	Zhao et al. 2022
	<i>Aporrectodea trapezoides</i>	PQ572753	15,014	this study
		OM687882	14,998	Zhao et al. 2022
	<i>Aporrectodea tuberculata</i>	OL840317	15,058	Zhao et al. 2022
		OM687883	15,125	Zhao et al. 2022
		OM687884	15,126	Zhao et al. 2022
		OM687885	15,129	Zhao et al. 2022
		OM687886	15,116	Zhao et al. 2022
	<i>Bimastus parvus</i>	MZ857199	15,194	Liu et al. 2021
	<i>Dendrobaena octaedra</i>	MZ857197	15,715	Liu et al. 2021
	<i>Eisenia andrei</i>	OK513069	15,714	Csuzdi et al. 2022
	<i>Eisenia fetida</i>	OK513070	16,560	Csuzdi et al. 2022
	<i>Eisenia nordenskiöldi</i>	MZ857200	15,152	Liu et al. 2021
		OM687887	16,114	Zhao et al. 2022
		OL840314	15,290	Zhao et al. 2022
	<i>Lumbricus rubellus</i>	MN102127	15,464	Zhang et al. 2019
	<i>Lumbricus terrestris</i>	U24570	14,998	Boore and Brown 1995
	<i>Octolasion tyrtaeum</i>	MZ857201	14,977	Liu et al. 2021
Moniligastridae	<i>Drawida japonica</i>	KM199288	14,648	Zhang et al. 2016
Rhinodrilidae	<i>Pontoscolex corethrurus</i>	KT988053	14,835	Conrado et al. 2017

Results

Based on the analysis of the barcoding region of *cox1* gene, our newly sequenced *Ap. trapezoides* specimen belongs to the widely distributed lineage 2 (Fernández et al. 2011). The *Ap. caliginosa* specimens analyzed were nested among the *caliginosa* lineage 2 specimens (Porco et al. 2013; Shekhovtsov et al. 2016), together with all *Ap. tuberculata* and *Ap. caliginosa* samples reported in the recent study by Zhao et al. (2022) (Fig. 1).

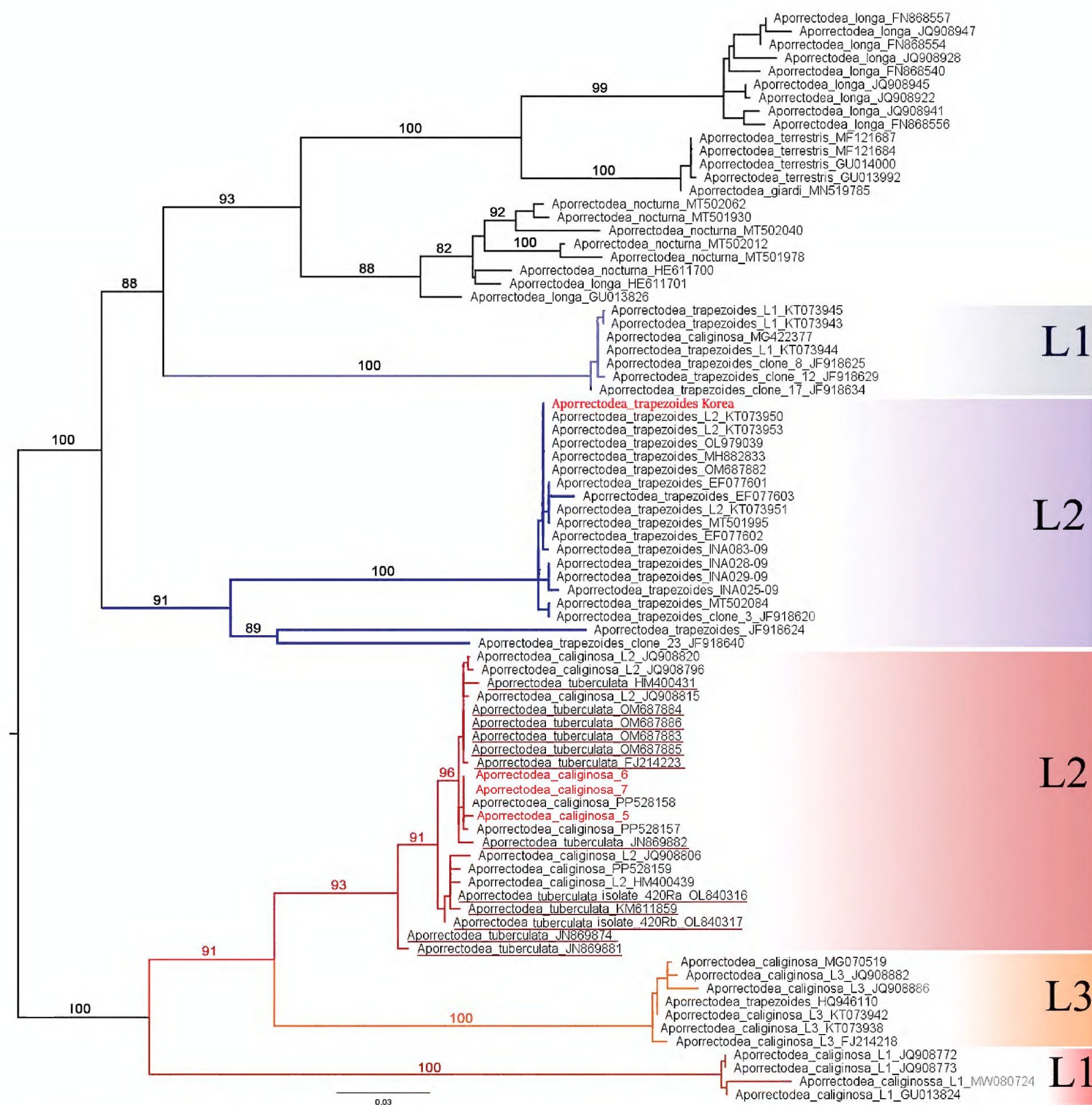


Figure 1. Maximum-likelihood (ML) tree based on the *cox1* gene fragments with bootstrap values of selected *Aporrectodea caliginosa* group specimens. Red names represent new sequences. L1, L2, and L3 refer to different lineages. *Ap. tuberculata* sequences are underlined.

The complete mt genome of *Ap. trapezoides* comprises 15,014 bp. The length of the three *Ap. caliginosa* mt genomes analyzed in this study varied between 15,090 and 15,123 bp. The mitogenome setup of both species followed the typical bauplan of the earthworm mitogenome assembly, consisting of 13 PCGs, 22 transfer RNAs, two ribosomal RNA genes, and a control region (Fig. 2; Table 2).

All genes were encoded on the heavy DNA strand, and both genomes showed biased base composition, with 63.5% AT and 36.4% GC content in *Ap. trapezoides* and 62.8% and 37.2% in *Ap. caliginosa*.

Overall mitogenome sequence similarity between the two species (*Ap. trapezoides* and *Ap. caliginosa* no. 5) was 80.8% and it increased to 85.8% when the control region was excluded. The 13 PCGs showed 71.2–85.9% similarity (Table 2). Among the PCGs, *cox2* showed the highest similarity (85.9%) and *atp8* the lowest (71.2%). The average similarity of the 13 PCGs between the two species was 84%. However, the deduced amino acid sequences of the 13 PCGs showed 92.7% similarity on average across species; *cox1* was the most similar (99.4%), and *atp8* the most dissimilar (66.04%; Table 3). The sequence variation between the two species was lower at the amino acid level than at the DNA level. In particular, *cox1* showed 82.47–84.41% similarity at the DNA level, but >99% similarity at the amino acid level (Table 4).

Phylogenetic reconstruction of the available complete or nearly complete lumbricid mitogenomes and use of the 13 PCGs strongly supported the family Lumbricidae (100% bootstrap support). In addition, the genera *Eisenia* and *Lumbricus* were resolved as monophyletic, and the close relationship between *Ap. trapezoides/caliginosa* species pairs was confirmed (Figs 3–5). Consequently, the genus *Aporrectodea* was found to be polyphyletic, as *Ap. rosea* did not form a clade with the other analyzed *Aporrectodea* species.

The uncorrected *p*-distances of the mitogenomes (excluding the non-coding region) between *Ap. caliginosa/tuberculata* and *Ap. trapezoides* ranged from 16.1% to 16.4% (Table 5). The two *Ap. trapezoides* (from China and Korea) did not exhibit any genetic distance (0.00%).

The maximum genetic distance within *Ap. caliginosa* was 11.1% between a Hungarian specimen (Apc-5) and [CM035405](#) (Azores Island, Portugal); however, between the L2 specimens identified as *Ap. tuberculata* and *Ap. caliginosa* it was only 2.1% (between Apc-5 and [OL840317](#)).

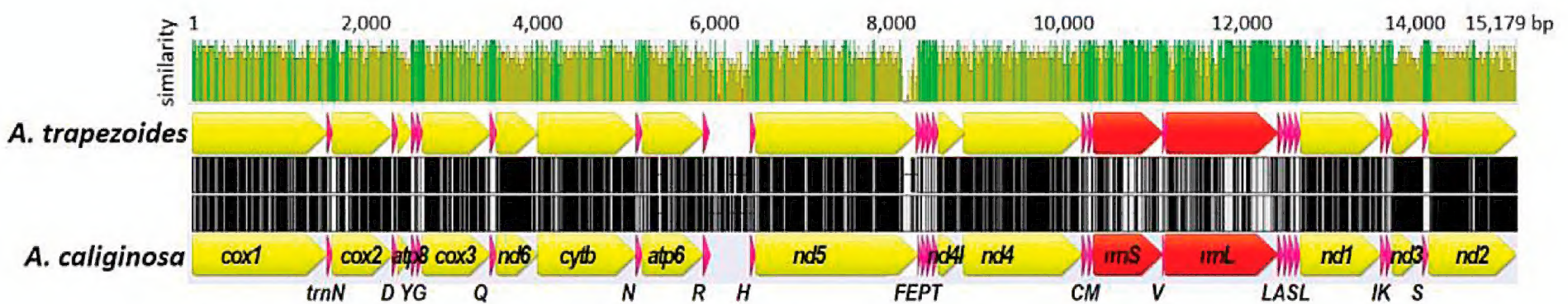


Figure 2. Comparison of the mitogenomes of *Aporrectodea trapezoides* and *Ap. caliginosa* (sample no. 5). Map was constructed based on sequence similarity using Geneious Prime 2021 software. Sequence similarity is represented by green (100%), brown (30–99%), and red (<30%). Yellow, pink, and red arrows indicate PCGs, tRNAs, and rRNAs, respectively. Organization of mitochondrial genes is described in Table 2. Non-coding (nc) region is defined between *trnR* and *trnH*.

Table 2. Comparative analysis of gene organization of *Aporrectodea trapezoides* and *Ap. caliginosa* mitogenomes.

Gene	<i>Ap. caliginosa</i> (CM035405)		<i>Ap. caliginosa</i> (NC_066400)		<i>Ap. tuberculata</i> (OL840317)		<i>Ap. trapezoides</i> (OM687882)		<i>Ap. trapezoides</i> (this study)		<i>Ap. caliginosa</i> 5 (this study)		Similarity (%)*
	Size (bp)	start/stop codon	Size (bp)	start/stop codon	Size (bp)	start/stop codon	Size (bp)	start/stop codon	Size (bp)	start/stop codon	Size (bp)	start/stop codon	
<i>cox1</i>	1540	ATG/T	1540	ATG/T	1540	ATG/T	1540	ATG/T	1540	ATG/T	1540	ATG/T	84.50%
<i>trnN</i>	61	–	61	–	61	–	61	–	61	–	61	–	88.9
<i>cox2</i>	687	ATG/TAG	687	ATG/TAG	687	ATG/TAG	687	ATG/TAG	687	ATG/TAG	687	ATG/TAG	85.9
<i>trnD</i>	61	–	61	–	61	–	61	–	61	–	61	–	90.2
<i>atp8</i>	160	ATG/T	160	ATG/T	160	ATG/T	160	ATG/T	160	ATG/T	160	ATG/T	71.2
<i>trnY</i>	63	–	63	–	63	–	62	–	62	–	63	–	95.2
<i>trnG</i>	63	–	63	–	63	–	63	–	63	–	63	–	93.7
<i>cox3</i>	778	ATG/T	778	ATG/T	778	ATG/T	778	ATG/T	778	ATG/T	778	ATG/T	83.8
<i>trnQ</i>	69	–	69	–	69	–	69	–	69	–	69	–	100
<i>nad6</i>	468	ATG/TAA	468	ATG/TAA	468	ATG/TAA	468	ATG/TAA	468	ATG/TAA	468	ATG/TAA	77.8
<i>cytb</i>	1140	ATG/TAA	1140	ATG/TAA	1140	ATG/TAA	1140	ATG/TAA	1140	ATG/TAA	1140	ATG/TAA	83
<i>trnW</i>	64	–	64	–	65	–	62	–	62	–	65	–	89.2
<i>atp6</i>	696	ATG/TAA	696	ATG/TAA	696	ATG/TAA	696	ATG/TAA	696	ATG/TAA	696	ATG/TAA	80.4
<i>trnR</i>	63	–	63	–	63	–	65	–	65	–	63	–	80.6
#NC	437	–	407	–	374	–	440	–	456	–	409	–	61
<i>trnH</i>	62	–	62	–	62	–	62	–	62	–	62	–	82.8
<i>nad5</i>	1722	ATG/TAA	1722	ATG/TAA	1722	ATG/TAA	1722	ATG/TAA	1722	ATG/TAA	1722	ATG/TAG	79.6
<i>trnF</i>	61	–	61	–	61	–	61	–	61	–	61	–	91.8
<i>trnE</i>	63	–	63	–	63	–	63	–	63	–	63	–	92.3
<i>trnP</i>	64	–	64	–	64	–	64	–	64	–	64	–	90.8
<i>trnT</i>	63	–	63	–	64	–	63	–	63	–	63	–	93.7
<i>nad4L</i>	297	ATG/TAA	297	ATG/TAA	297	ATG/TAA	297	ATG/TAA	297	ATG/TAA	297	ATG/TAA	79.9
<i>nad4</i>	1359	ATG/TAG	1359	ATG/TAG	1359	ATG/TAG	1350	ATG/TAG	1359	ATG/TAG	1359	ATG/TAG	80.8
<i>trnC</i>	65	–	65	–	65	–	65	–	65	–	65	–	90.9
<i>trnM</i>	63	–	63	–	63	–	63	–	63	–	63	–	96.8
<i>rrnS</i>	788	–	788	–	788	–	789	–	795	–	794	–	89
<i>trnV</i>	63	–	63	–	63	–	63	–	63	–	63	–	93.8
<i>rrnL</i>	1257	–	1256	–	1256	–	1256	–	1275	–	1295	–	86
<i>trnL</i>	62	–	62	–	62	–	62	–	62	–	62	–	93.7
<i>trnA</i>	62	–	62	–	62	–	62	–	62	–	62	–	88.7
<i>trnS</i>	67	–	67	–	67	–	67	–	67	–	67	–	94.1
<i>trnL</i>	62	–	62	–	62	–	62	–	62	–	62	–	98.4
<i>nad1</i>	922	ATG/T	922	ATG/T	922	ATG/T	922	ATG/T	922	ATG/T	922	ATG/T	83.6
<i>trnI</i>	66	–	66	–	66	–	64	–	64	–	66	–	90.9
<i>trnK</i>	63	–	63	–	63	–	64	–	64	–	63	–	92.3
<i>nad3</i>	354	ATG/TAG	354	ATG/TAG	354	ATG/TAG	354	ATG/TAG	354	ATG/TAG	354	ATG/TAG	74.6
<i>trnS</i>	64	–	64	–	64	–	64	–	64	–	64	–	100
<i>nad2</i>	1006	ATG/T	1006	ATG/T	1006	ATG/T	1006	ATG/T	1006	ATG/T	1006	ATG/T	79.1

#Putative non-coding region between *trnR* and *trnH*.
* indicates the similarity of nucleotide sequence between *Ap. trapezoides* and *Ap. caliginosa* (Apc-5).

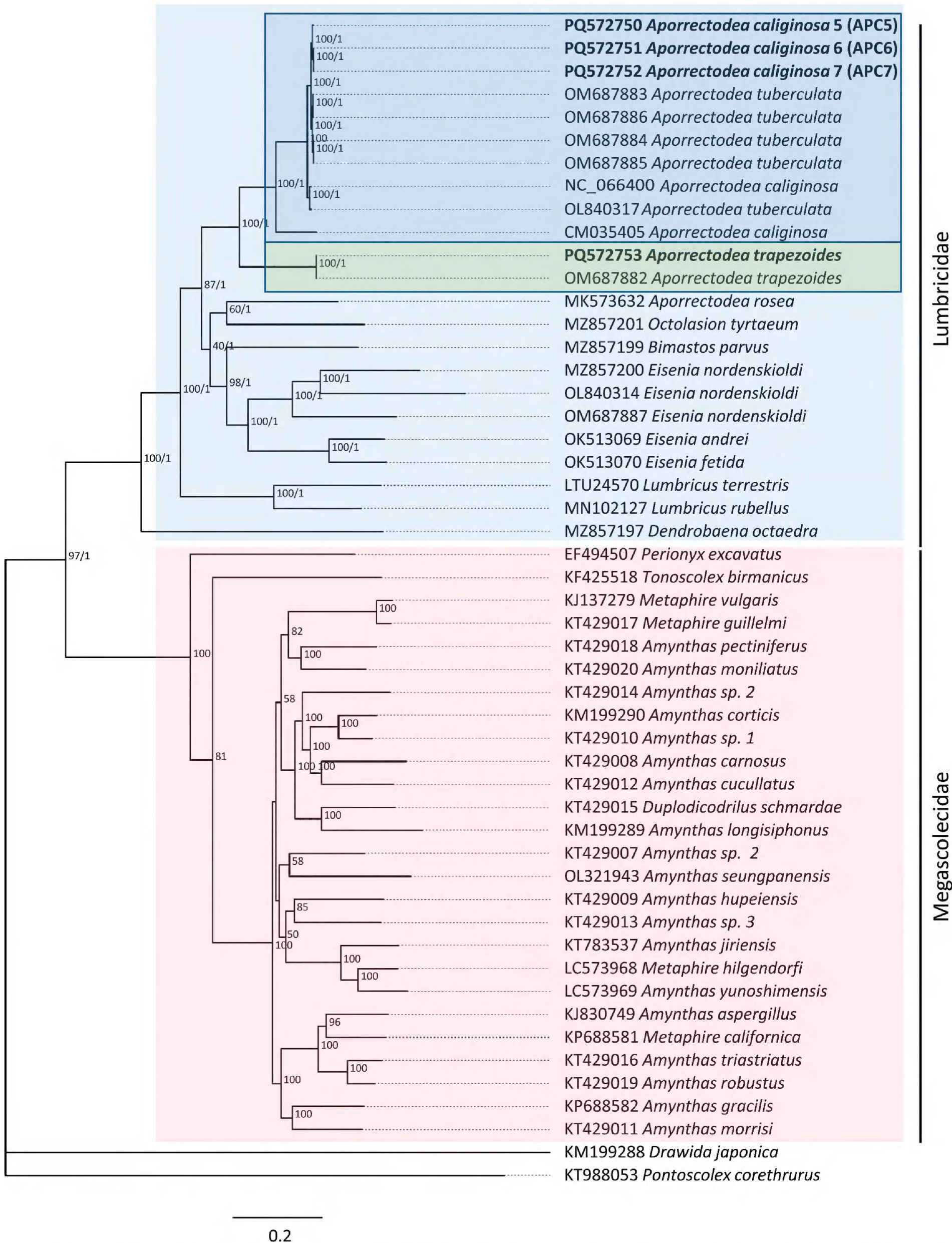


Figure 3. Phylogenetic analysis of 40 Megadrili group spp., including *Aporrectodea trapezoides* and *Ap. caliginosa*, based on sequences of 37 mitochondrial genes. Numbers beside nodes are ML bootstrap values and BI posterior probabilities. *Drawida japonica* and *Pontoscolex corethrurus* were included as outgroups.

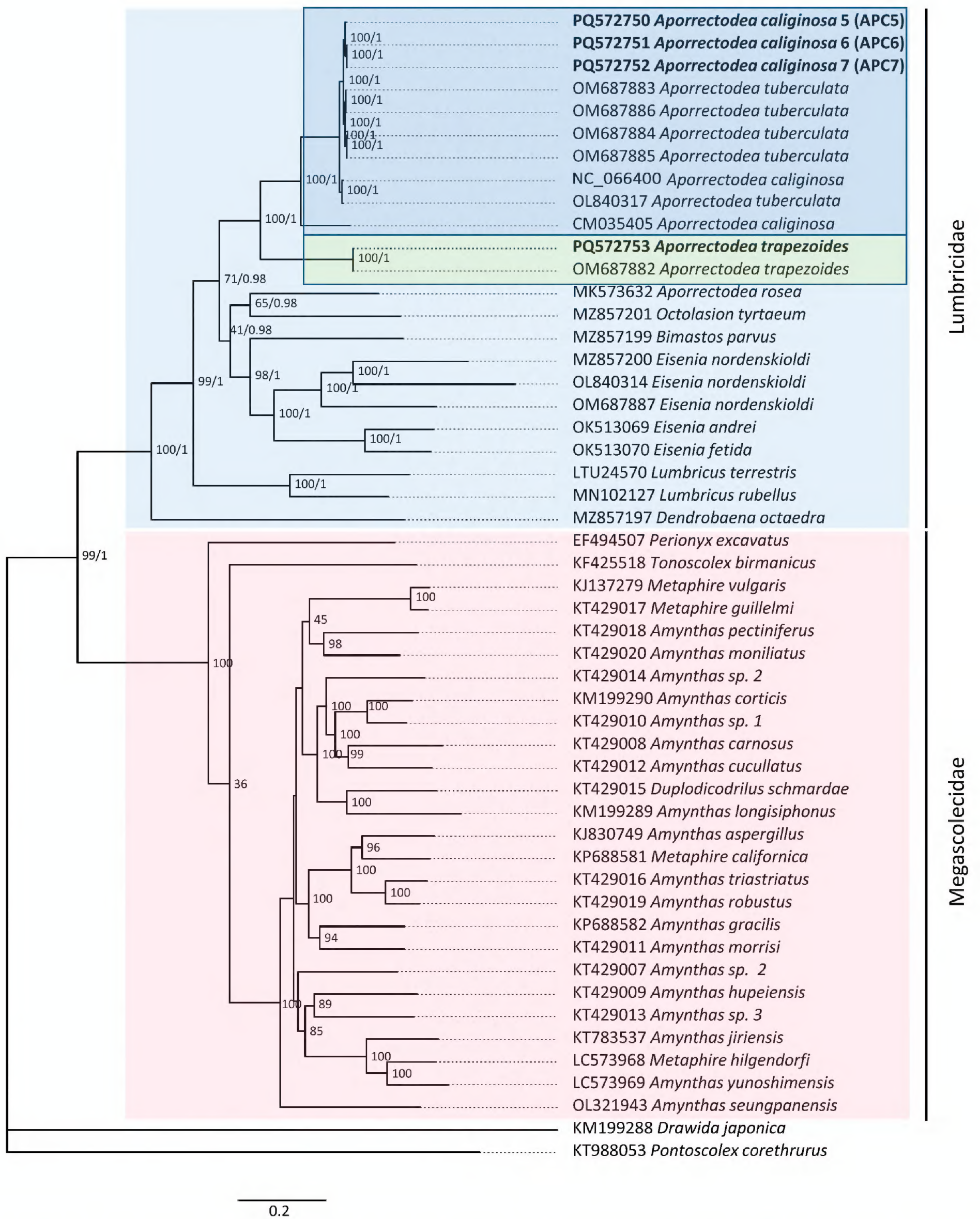


Figure 4. Phylogenetic analysis of 40 Megadrili group spp. using 13 PCGs, including *Aporrectodea trapezoides* and *Ap. caliginosa*. Numbers beside nodes are ML bootstrap values and BI posterior probabilities. *Drawida japonica* and *Pontoscolex corethrurus* were included as outgroups.

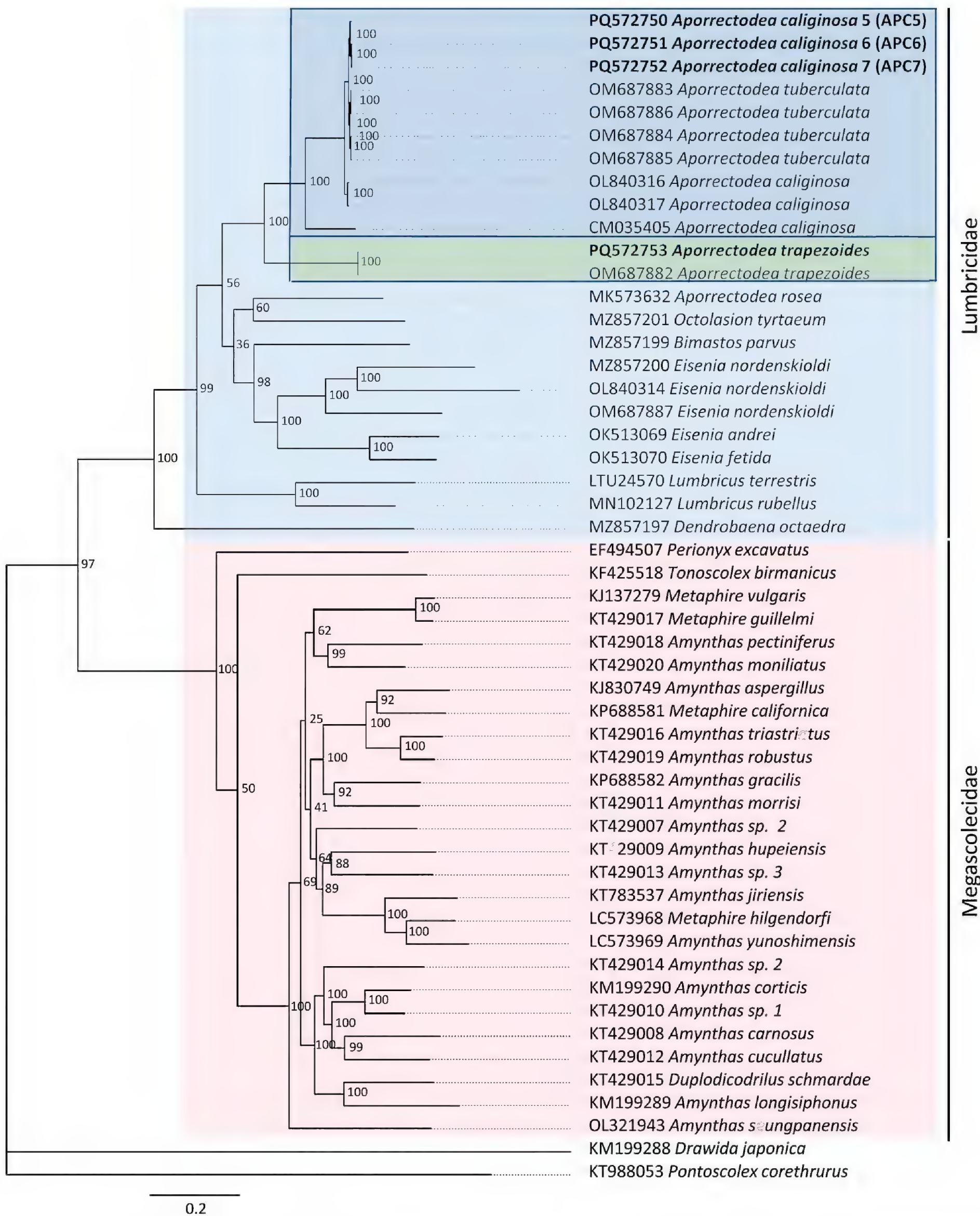


Figure 5. Partitioned phylogenetic analysis of 40 Megadrili group spp. using 13 PCGs, including *Aporrectodea trapezoides* and *Ap. caliginosa*. Numbers beside nodes are ML bootstrap values.

Table 3. Comparison of deduced amino acid composition of 13 PCGs in the *Aporrectodea caliginosa* species group.

		<i>Ap. trapezoides</i> this study vs.	<i>Ap. trapezoides</i> this study vs.	<i>Ap. trapezoides</i> this study vs.	<i>Ap. caliginosa</i> (CM035405) vs.	<i>Ap. caliginosa</i> (Apc-5) vs.
Protein	aa size	<i>A. caliginosa</i> (CM035405)	<i>A. caliginosa</i> (NC_066400)	<i>A. caliginosa</i> (Apc-5)	<i>A. caliginosa</i> (Apc-5)	<i>A. tuberculata</i> (OM687883)
COX1	513	99.40%	99.40%	99.40%	100%	100%
COX2	228	97.20%	96.73%	96.73%	99.53%	100%
ATP8	53	66.04%	66.04%	67.92%	86.79%	96.23%
COX3	259	96.00%	95.60%	95.60%	98.00%	99.60%
NAD6	156	86.71%	88.59%	86.58%	95.10%	97.99%
CYTB	379	97.35%	94.99%	95.78%	97.88%	99.74%
ATP6	231	89.96%	89.52%	89.52%	95.20%	99.13%
NAD5	573	91.94%	91.97%	91.97%	97.55%	99.30%
NAD4L	98	97.50%	91.84%	92.86%	92.86%	100%
NAD4	452	90.78%	91.57%	91.59%	97.00%	99.12%
NAD1	307	93.20%	94.77%	95.11%	95.58%	99.67%
NAD3	117	92.71%	88.79%	86.32%	94.79%	96.58%
NAD2	335	89.55%	87.74%	88.42%	95.82%	99.04%

* All 13 mitochondrial proteins from mitogenome showed same size across the species.

Table 4. Comparison of the nucleotide sequences of the 13 PCGs between *Ap. trapezoides* and *Ap. caliginosa*.

	<i>Ap. trapezoides</i> this study vs.	<i>Ap. trapezoides</i> this study vs.	<i>Ap. trapezoides</i> this study vs.	<i>A. caliginosa</i> (CM035405) vs.	<i>A. caliginosa</i> 5 this study vs.
Protein	<i>A. caliginosa</i> (CM035405)	<i>A. caliginosa</i> (NC_066400)	<i>A. caliginosa</i> 5 this study	<i>A. caliginosa</i> 5 this study	<i>A. tuberculata</i> (OM687883)
COX1	82.47%	84.93%	84.41%	88.05%	99.03%
COX2	85.20%	85.96%	85.82%	89.88%	99.12%
ATP8	74.36%	73.58%	72.33%	84.62%	98.74%
COX3	83.20%	84.04%	83.53%	88.53%	99.49%
NAD6	79.87%	78.49%	77.63%	84.12%	98.49%
CYTB	82.94%	83.03%	82.76%	86.74%	99.03%
ATP6	80.06%	79.80%	80.23%	86.03%	98.70%
NAD5	79.04%	79.76%	79.64%	87.10%	98.78%
NAD4L	78.75%	79.93%	79.59%	82.50%	100%
NAD4	91.56%	80.68%	80.68%	86.28%	98.67%
NAD1	82.16%	84.69%	83.93%	86.73%	98.59%
NAD3	77.78%	76.92%	76.07%	84.05%	97.72%
NAD2	78.92%	78.81%	79.20%	85.64%	99.10%

Discussion

Here, we report the first complete mitogenomes of *Ap. caliginosa* and *Ap. trapezoides*. The newly analyzed mt genomes showed an arrangement and base composition typical of earthworms (Shekhovtsov et al. 2020; Csuzdi et al. 2022) and fit into the size distribution of previously known complete earthworm mitogenomes (14,648–16,560) (Csuzdi et al. 2022; Zhao et al. 2022). Our data are in agreement with the published incomplete *Ap. caliginosa/tuberculata* mitogenomes (15,058–15,129 vs 15,090–15,123) which demonstrates that the

Table 5. Genetic *p*-distances of selected Lumbricidae mitogenomes using the complete mitogenome sequences excluding the non-coding region.

<i>Ap. rosea</i> MK573632												
<i>Ap. trapezoides</i> OM687882	0.203											
<i>Ap. trapezoides</i> this study	0.203	0.000										
<i>Ap. caliginosa</i> CM035405	0.202	0.164	0.163									
<i>Ap. caliginosa</i> NC_066400	0.201	0.161	0.161	0.110								
<i>Ap. caliginosa</i> (Apc-5)	0.200	0.164	0.164	0.111	0.021							
<i>Ap. caliginosa</i> (Apc-6)	0.201	0.164	0.164	0.110	0.020	0.004						
<i>Ap. caliginosa</i> (Apc-7)	0.201	0.164	0.164	0.110	0.020	0.004	0.000					
<i>Ap. tuberculata</i> OL840317	0.200	0.161	0.161	0.109	0.006	0.021	0.020	0.020				
<i>Ap. tuberculata</i> OM687884	0.201	0.163	0.163	0.109	0.020	0.011	0.010	0.010	0.020			
<i>Ap. tuberculata</i> OM687885	0.201	0.163	0.163	0.109	0.020	0.011	0.010	0.010	0.020	0.000		
<i>Ap. tuberculata</i> OM687883	0.201	0.163	0.163	0.109	0.020	0.010	0.009	0.009	0.020	0.005	0.005	
<i>Ap. tuberculata</i> OM687886	0.201	0.163	0.163	0.109	0.020	0.010	0.009	0.009	0.020	0.005	0.005	0.000

data from Zhao et al. (2022) are nearly complete, and only a few bp may be missing for circularity. For *Ap. trapezoides* (14,998 vs 15,014), this difference was also negligible.

Amino acid and base pair compositions across the 13 PCGs in *Ap. caliginosa* L2 and *Ap. tuberculata* species pairs were highly similar, showing 96–100% similarity across different genes. We obtained slightly lower similarity data when we compared *Ap. caliginosa* L2, and the sequence belonged to L3 ([CM035405](#)), indicating that the *Ap. tuberculata* sequences reported by Zhao et al. (2022) belong to *Ap. caliginosa*. This was clearly depicted in the phylogenetic reconstructions using the *cox1*, 13 PCGs, and complete mt genomes (Figs 1, 3, 4), corroborating the earlier findings of Briard et al. (2012) and Fernández et al. (2012) that *Ap. tuberculata* auct. (Eisen, 1874) is a synonym for *Ap. caliginosa* (Savigny, 1826). However, this was not observed for *Ap. trapezoides*, corroborating with previous results (Pérez-Losada et al. 2009; Fernández et al. 2011, 2012) and suggesting that *Ap. trapezoides* is distant from *Ap. caliginosa*, as it showed only 72.33%–91.56% nucleotide sequence similarity and 86.32%–99.4% amino acid composition similarity (lowest for *nad3* and highest for *cox1*; Tables 3, 4). This result was also reflected in the phylogenetic trees (Figs 1, 3, 4).

Phylogenetic analysis of the complete mt genomes (excluding the control region) and 13 PCGs strongly supported the monophyletic family Lumbricidae; however, the genus *Aporrectodea* proved to be polyphyletic. The species *Ap. rosea* does not form a clade with *Ap. caliginosa*/*trapezoides* sequences, reinforcing findings of previous studies’ (Domínguez et al. 2015; Marchán et al. 2022, 2023), and restricting *Aporrectodea* Örley, 1885 to the *Ap. caliginosa*/*trapezoides* complex and other closely related Franco-Iberian species (Marchán et al. 2023).

Acknowledgements

We would like to thank Editage (www.editage.co.kr) for English language editing. We thank Jovana Seculic, Parin Jirapatrasilp and an anonymous reviewer for their valuable comments on the manuscript.

Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

The samples used in this study are earthworms that are not included in the list of protected animals, and hence, the ethical statement is not applicable.

Funding

This study was supported by the National Research Foundation of Korea (NRF) funded by the Ministry of Education under Grant number RS-2024-00338212.

Author contributions

Conceptualization: YH. Data curation: JK. Formal analysis: CC, JK. Funding acquisition: YH. Methodology: JK. Writing – original draft: CC. Writing – review and editing: YH.

Author ORCIDs

Csaba Csuzdi  <https://orcid.org/0000-0002-0319-7836>

Jachoon Koo  <https://orcid.org/0000-0002-3559-326X>

Yong Hong  <https://orcid.org/0000-0002-8093-9717>

Data availability

The genome sequence data supporting the results of this study are openly available in GenBank of NCBI under the accession no. [PQ572750–PQ572753](#).

References

- Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA (2012) Spades: A new genome assembly algorithm and its applications to single-cell sequencing. *Journal of Computational Biology* 19: 455–477. <https://doi.org/10.1089/cmb.2012.0021>
- Blakemore RJ (2002) *Cosmopolitan earthworms – an ecotaxonomic guide to the peregrine species of the world*, VermEcology Kippax ACT Australia, 506 pp.
- Blakemore RJ (2008) *Cosmopolitan earthworms – an eco-taxonomic guide to the species*. 3rd edition. VermEcology Yokohama, 757 pp.
- Bolger AM, Lohse M, Usade, B (2014) Trimmomatic – a flexible trimmer for Illumina sequence data. *Bioinformatics* 30(15): 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>
- Boore JL, Brown WM (1995) Complete sequence of the mitochondrial DNA of the annelid worm *Lumbricus terrestris*. *Genetics* 141(1): 305–319. <https://doi.org/10.1093/genetics/141.1.305>
- Bouché BM (1972) *Lombriciens de France Écologie et Systématique*. Institut National de la Recherche Agronomique. Annales de Zoologie Ecologie Animale. Numéro hors-série, Paris, 671 pp.
- Briard C, Qiu J-P, Zhao Q, Guernion M, Cluzeau D (2012) Phylogenetic study of some *Aporrectodea* species based on molecular markers. *Zoology in the Middle East* 58(Supplementum 4): 23–30. <https://doi.org/10.1080/09397140.2012.10648982>

- Briones MJ (1996) A taxonomic revision of the *Allolobophora caliginosa* complex (Oligochaeta, Lumbricidae): a preliminary study. *Canadian Journal of Zoology* 74(2): 240–244. <https://doi.org/10.1139/z96-030>
- Cain AJ (1955) The taxonomic statues of *Allolobophora iowana* Evans, 1948 (Oligochaeta, Lumbricidae). *Annals and Magazine of Natural History (Series 12)* 8(91): 481–497. <https://doi.org/10.1080/00222935508655659>
- Capella-Gutiérrez S, Silla-Martínez JM, Gabaldón T (2009) trimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics* 25(15): 1972–1973. <https://doi.org/10.1093/bioinformatics/btp348>
- Conrado AC, Arruda H, Stanton DWG, James SW, Kille P, Brown G, Silva E, Dupont L, Taheri S, Morgan AJ, Simões N, Rodrigues A, Montiel R, Cunha L (2017) The complete mitochondrial DNA sequence of the pantropical earthworm *Pontoscolex corethrurus* (Rhinodrilidae, Clitellata): mitogenome characterization and phylogenetic positioning. *ZooKeys* 688: 1–13. <https://doi.org/10.3897/zookeys.688.13721>
- Csuzdi Cs, Zicsi A (2003) Earthworms of Hungary (Annelida: Oligochaeta, Lumbricidae). Hungarian Natural History Museum, Budapest, 273 pp. <https://doi.org/10.5281/zenodo.4309820>
- Csuzdi Cs, Koo J, Hong Y (2022) The complete mitochondrial DNA sequences of two sibling species of lumbricid earthworms, *Eisenia fetida* (Savigny, 1826) and *Eisenia andrei* (Bouché, 1972) (Annelida, Crassiciellata): comparison of mitogenomes and phylogenetic positioning. *ZooKeys* 1097: 167–181. <https://doi.org/10.3897/zookeys.1097.80216>
- Domínguez J, Aira M, Breinholt JW, Stojanovic M, James SW, Pérez-Losada M (2015) Underground evolution: new roots for the old tree of lumbricid earthworms. *Molecular Phylogenetics and Evolution* 83: 7–19. <https://doi.org/10.1016/j.ympev.2014.10.024>
- Donath A, Jühling F, Al-Arab M, Bernhar, SH, Reinhardt F, Stadler PF, Middendorf M, Berrnt M (2019) Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *Nucleic Acids Research* 47(20): 10543–10552. <https://doi.org/10.1093/nar/gkz833>
- Dugès A (1828) Recherches sur la circulation, la respiration et la reproduction des annelides abranches. *Annales des Sciences Naturelles* 15: 284–337.
- Eisen G (1873) Om Skandinaviens Lumbricier. Ofversigt af Kongliga Vetenskaps-Akademiens Forhandlingar 1873(8): 43–56. <https://archive.org/details/biostor-135527>
- Eisen G (1874) Bidrag till kännedomen om New Englands och Canadas Lumbricider. Ofversigt af Kongliga Vetenskaps-Akademiens Forhandlingar 1874(2): 41–50.
- Fernández R, Almodóvar A, Novo M, Gutiérrez M, Díaz Cosín DJ (2011) A vagrant clone in a peregrine species: phylogeography, high clonal diversity and geographical distribution in the earthworm *Aporrectodea trapezoides* (Dugès, 1828). *Soil Biology and Biochemistry* 43(10): 2085–2093. <https://doi.org/10.1016/j.soilbio.2011.06.007>
- Fernández R, Almodóvar, A, Novo M, Simancas B, Díaz Cosín DJ (2012) Adding complexity to the complex: new insights into the phylogeny, diversification and origin of parthenogenesis in the *Aporrectodea caliginosa* species complex (Oligochaeta, Lumbricidae). *Molecular Phylogenetics and Evolution* 64(2): 368–379. <https://doi.org/10.1016/j.ympev.2012.04.011>
- Fernández R, Almodóvar A, Novo M, Gutiérrez M, Díaz Cosín DJ (2013) Earthworms, good indicators for palaeogeographical studies? Testing the genetic structure and demographic history in the peregrine earthworm *Aporrectodea trapezoides* (Dugès, 1828) in southern Europe. *Soil Biology and Biochemistry* 58: 127–135. <https://doi.org/10.1016/j.soilbio.2012.10.021>
- Friend H (1910) New garden worms. *Gardener's chronicle* 48: 98–99.

- Gates GE (1972a) Burmese earthworms. An introduction to the systematics of megadrile oligochaetes with special references to Southeast Asia. Transactions of the American Philosophical Society 62: 1–326. <https://doi.org/10.2307/1006214>
- Gates GE (1972b) Toward a revision of the earthworm family Lumbricidae. IV. The *trapezoides* species group. Bulletin of the Tall Timbers Research Station 12: 1–146 pp.
- Hoang, DT, Chernomor O, von Haeseler A, Minh BQ, Vinh LS (2018) UFBoot2: Improving the ultrafast bootstrap approximation. Molecular Biology and Evolution 35: 518–522. <https://doi.org/10.1093/molbev/msx281>
- Hong Y, Kim MJ, Wang AR, Kim I (2017) Complete mitochondrial genome of the earthworm, *Amyntas jiriensis* (Clitellata: Megascolecidae). Mitochondrial DNA A. DNA Mapping, Sequencing, and Analysis 28(2):163–164. <https://doi.org/10.3109/19401736.2015.1115491>
- Kalyaanamoorthy S, Minh BQ, Wong TKF, von Haesele, A, Jermiin LS (2017) ModelFinder: fast model selection for accurate phylogenetic estimates. Nature Methods 14(6): 587–589. <https://doi.org/10.1038/nmeth.4285>
- Katoh K, Standley DM (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30(4): 772–780. <https://doi.org/10.1093/molbev/mst010>
- Kim MJ, Hong Y (2022) Complete mitochondrial genome of the earthworm *Amyntas seungpanensis* (Clitellata: Megascolecidae). Mitochondrial DNA B Resources 7(6): 989–991. <https://doi.org/10.1080/23802359.2022.2080604>
- Kumar S, Stecher G, Li M, Knyaz C, Tamura K (2018) MEGA X: Molecular Evolutionary Genetics Analysis across computing platforms. Molecular Biology and Evolution 35(6): 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Latif R, Malek M, Aminjan AR, Pasantes JJ, Briones MJ, Csuzdi CS (2020) Integrative taxonomy of some Iranian peregrine earthworm species using morphology and barcoding (Annelida: Megadrili). Zootaxa 4877(1): 163–173. <https://doi.org/10.11646/zootaxa.4877.1.7>
- Liu H, Zhang Y, Xu W, Fang Y, Ruan H (2021) Characterization of five new earthworm mitogenomes (Oligochaeta: Lumbricidae): mitochondrial phylogeny of Lumbricidae. Diversity 13(11): 580. <https://doi.org/10.3390/d13110580>
- Marchán DF, Hedde M, Lapied E, Maggia ME, Novo M, Domínguez J, Decaëns T (2020) Contrasting phylogeographic patterns of earthworms (Crassicitellata, Lumbricidae) on near-shore mediterranean islands. European Journal of Soil Biology 101: 103242. <https://doi.org/10.1016/j.ejsobi.2020.103242>
- Marchán DF, James SW, Lemmon AR, Moriarty Lemon E, Novo M, Domínguez J, Diaz Cosin DJ, Trigo D (2022) A strong backbone for an invertebrate group: anchored phylogenomics improves the resolution of genus-level relationships within the Lumbricidae (Annelida, Crassicitellata). Organisms Diversity & Evolution 22: 915–924. <https://doi.org/10.1007/s13127-022-00570-y>
- Marchán DF, Navarro AM, Pinadero SJ, Gerard S, Hedde M, Domínguez J, Decaëns T, Novo M (2023) Understanding the diversification and functional radiation of *Aporrectodea* (Crassicitellata, Lumbricidae) through molecular phylogenetics of its endemic species. European Journal of Soil Biology 119: 103559. <https://doi.org/10.1016/j.ejsobi.2023.103559>
- Michaelsen W (1892) Terricolen der Berliner Zoologischen Sammlung, II. Archiv für Naturgeschichte 58: 209–261. <https://doi.org/10.5962/bhl.part.8321>
- Mršić N (1991) Monograph on earthworms (Lumbricidae) of the Balkans I–II. Slovenska Akademija Znanosti in Umetnosti, Razred za Naravoslovne Vede, Opera, 31, Ljubljana, 757 pp.

- Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ (2014) IQ-TREE: a fast and effective stochastic algorithm for Estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution* 32(1): 268–274. <https://doi.org/10.1093/molbev/msu300>
- Örley L (1885) A palaearktikus övben élő terrikoláknak revíziója és elterjedése. *Értekezések a Természettudományok Köréből* 15: 1–34.
- Pérez-Losada, M, Ricoy M, Marshall JC, Domínguez J (2009) Phylogenetic assessment of the earthworm *Aporrectodea caliginosa* species complex (Oligochaeta: Lumbricidae) based on mitochondrial and nuclear DNA sequences. *Molecular Phylogenetics and Evolution* 52(2): 293–302. <https://doi.org/10.1016/j.ympev.2009.04.003>
- Pop V (1949) Lumbricidele din România. *Analele Academiei Republicii Populare Române* 1(9): 383– 505.
- Porco D, Decaëns T, Deharveng L, James SW, Skarżyński D, Erséus C, Butt KR, Richard B, Hebert PDN (2013) Biological invasions in soil: DNA barcoding as a monitoring tool in a multiple taxa survey targeting European earthworms and springtails in North America. *Biological Invasions* 15(4): 899–910. <https://doi.org/10.1007/s10530-012-0338-2>
- Ratnasingham S, Hebert PDN (2013) A DNA-based registry for all animal species: the Barcode Index Number (BIN) System. *PLoS ONE* 8(8): e66213. <https://doi.org/10.1371/journal.pone.0066213>
- Reynolds JW (1977) The earthworms (Lumbricidae and Sparganophilidae) of Ontario. Royal Ontario Museum Toronto, 141 pp. <https://doi.org/10.5962/bhl.title.60740>
- Reynolds JW (2022) The Earthworms (Lumbricidae, Megascolecidae and Sparganophilidae) in Canada. Canadian Food Inspection Agency Ottawa, 179 pp.
- Ronquist F, Teslenko M, van der Mark P, Ayres DL, Darling A, Höhna S, Larget B, Liu L, Suchard MA, Huelsenbeck JP (2012) MRBAYES 3.2: efficient Bayesian phylogenetic inference and model selection across a large model space. *Systematic Biology* 61: 539–542. <https://doi.org/10.1093/sysbio/sys029>
- Savigny JC (1826) Analyse d'un memoire sur les Lombrics par Cuvier. *Mémoires de l'Académie des sciences de l'Institut de France* 5: 176–184.
- Schwarz G (1978) Estimating the dimension of a model. *The Annals of Statistics* 6(2): 461–464. <https://doi.org/10.1214/aos/1176344136>
- Seto A, Endo H, Minamiya Y, Matsuda M (2021) The complete mitochondrial genome sequences of Japanese earthworms *Metaphire hilgendorfi* and *Amyntas yunoshimensis* (Clitellata: Megascolecidae). *Mitochondrial DNA B Resources* 6(3): 965–967. <https://doi.org/10.1080/23802359.2020.1830728>
- Shekhovtsov SV, Peltek SE (2019) The complete mitochondrial genome of *Aporrectodea rosea* (Annelida: Lumbricidae). *Mitochondrial DNA Part B, Resources* 4(1): 1752–1753. <https://doi.org/10.1080/23802359.2019.1610091>
- Shekhovtsov SV, Golovanova EV, Peltek SE (2016) Different dispersal histories of lineages of the earthworm *Aporrectodea caliginosa* (Lumbricidae, Annelida) in the Palearctic. *Biological Invasions* 18(3): 751–761. <https://doi.org/10.1007/s10530-015-1045-6>
- Shekhovtsov SV, Golovanova EV, Bazarova NE, Belova YN, Berman DI, Derzhinsky EA, Shashkov MP, Peltek SE (2017) Genetic diversity of the *Aporrectodea caliginosa* complex in Russia. *Vavilov Journal of Genetics and Breeding* 21(3): 374–379. <https://doi.org/10.18699/VJ17.255>
- Shekhovtsov SV, Golovanova EV, Ershov NI, Poluboyarova TV, Berman DI, Bulakhova NA, Szederjesi T, Peltek SE (2020) Phylogeny of the *Eisenia nordenskioldi* complex based on mitochondrial genomes. *European Journal of Soil Biology* 96: 103137. <https://doi.org/10.1016/j.ejsobi.2019.103137>

- Sims RW, Gerard BM (1985) Earthworms. Keys and notes to the identification and study of the Species. Synopsis of the British Fauna (New Series) E.J. Brill Leiden, 171 pp. <https://doi.org/10.1163/9789004611405>
- Templeton R (1836) A catalogue of the species of annulose animals, and of rayed ones, found in Ireland, as selected from the papers of the late J. Templeton, Esq., of Cranmore, with localities, description and illustration. Magazine of Natural History and Journal of Zoology, Botany, Mineralogy, Geology and Meteorology 9: 233–240.
- Tétry A (1937) Revision des lombriciens de la collection de Savigny. Bulletin du Muséum national d'Histoire naturelle 2(1): 72–81.
- Wang AR, Hong Y, Win TM, Kim I (2015) Complete mitochondrial genome of the Burmese giant earthworm, *Tonoscolex birmanicus* (Clitellata: Megascolecidae). Mitochondrial DNA. The Journal of DNA Mapping, Sequencing, and Analysis 26(3): 467–468. <https://doi.org/10.3109/19401736.2013.830300>
- Zhang L, Sechi P, Yuan M, Jiang J, Dong Y, Qiu J (2016) Fifteen new earthworm mitogenomes shed new light on phylogeny within the *Pheretima* complex. Scientific Reports 6: 20096. <https://doi.org/10.1038/srep20096>
- Zhao H, Fan S, Aspe NM, Feng L, Zhang (2022) Characterization of 15 earthworm mitogenomes from northeast China and its phylogenetic implication (Oligochaeta: Lumbricidae, Moniligastridae). Diversity 14(9): 714. <https://doi.org/10.3390/d14090714>
- Zicsi A (1982) Verzeichnis der bis 1971 beschriebenen und revidierten Taxa der Familie Lumbricidae (Oligochaeta). Acta zoologica hungarica 28: 421–454.